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(54) **TOBACCO INBRED PLANTS NCBEX1F, NCBEX1MS, AND NC EX90**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides tobacco inbred plants NCBEX1F and NCBEX1MS, and NC EX90. The present invention also provides parts of such plants and products made from those parts. The present invention also includes progeny of the provided plants including hybrids.

28 Claims, 2 Drawing Sheets

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FIG. 1

FIG. 2A

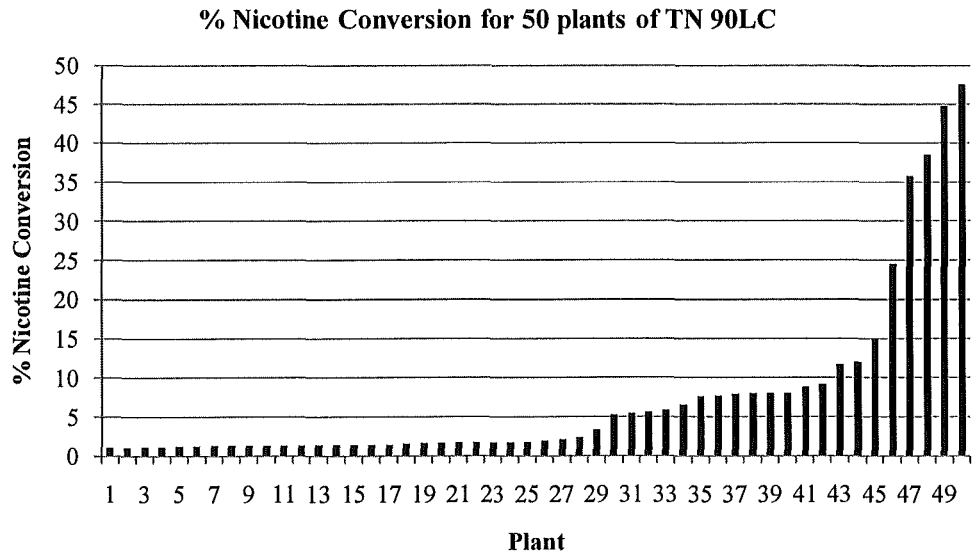
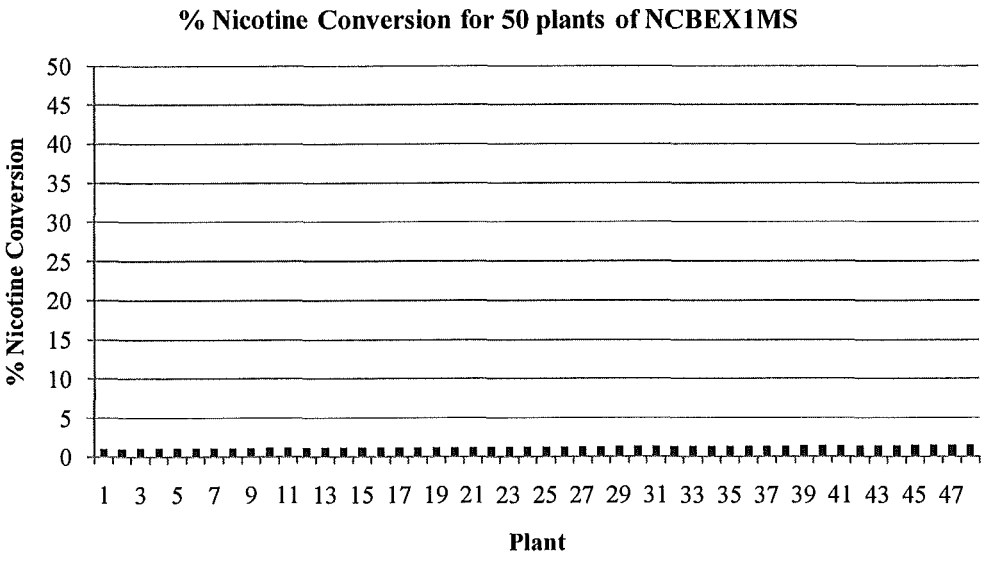

% Nicotine Conversion for 50 plants of TN 90LC
% Nicotine Conversion
50
45
40
35
30
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49
Plant

FIG. 2B


% Nicotine Conversion for 50 plants of NCBEX1MS
% Nicotine Conversion
50
45
40
35
30
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Plant

TOBACCO INBRED PLANTS NCBEX1F, NCBEX1MS, AND NC EX90

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119 of U.S. Provisional Application No. 61/447,443, filed Feb. 28, 2011 and U.S. Provisional Application No. 61/546,573, filed Oct. 13, 2011, each of which is herein incorporated by reference in its entirety, including its respective sequence listing.

INCORPORATION OF SEQUENCE LISTING

The Sequence Listing is hereby incorporated by reference in its entirety, including the file named P33831US02.txt, which is 28,672 bytes in size and was created on Feb. 17, 2012, which is likewise herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention provides tobacco inbred plants NCBEX1F, NCBEX1MS, and NC EX90. The present invention also provides parts of such plants and products made from those parts. The present invention also includes progeny of the provided plants including hybrids.

BACKGROUND OF THE INVENTION

Tobacco (*Nicotiana tabacum* L.) is an important commercial crop in the United States as well as in other countries. In tobacco plants, N-demethylation of nicotine results in nornicotine, a secondary alkaloid known to be a precursor for formation of N-Nitrosonornicotine ("NNN") in cured leaves. NNN is an undesired component of cured leaves.

The predominant alkaloid found in commercial tobacco varieties is nicotine, typically accounting for 90-95% of the total alkaloid pool. The remaining alkaloid fraction is comprised primarily of three additional pyridine alkaloids: nornicotine, anabasine, and anatabine. Nornicotine is generated directly from nicotine through the activity of the enzyme nicotine N-demethylase. Nornicotine usually represents less than 5% of the total pyridine alkaloid pool, but through a process termed "conversion," tobacco plants that initially produce very low amounts of nornicotine give rise to progeny that metabolically "convert" a large percentage of leaf nicotine to nornicotine. In tobacco plants that have genetically converted (termed "converters"), the great majority of nornicotine production occurs during the senescence and curing of the mature leaf (Wernsman and Matzinger (1968) *Tob. Sci.* 12:226-228). Burley tobaccos are particularly prone to genetic conversion, with rates as high as 20% per generation observed in some cultivars.

During the curing and processing of the tobacco leaf, a portion of the nornicotine is metabolized to the compound NNN, a tobacco-specific nitrosamine (TSNA) that has been asserted to be carcinogenic in laboratory animals (Hecht and Hoffmann (1990) *Cancer Surveys* 8:273-294; Hoffmann et al. (1994) *J. Toxicol. Environ. Health* 41:1-52; Hecht (1998) *Chem. Res. Toxicol.* 11:559-603). In flue-cured tobaccos, TSNAs are found to be predominantly formed through the reaction of alkaloids with the minute amounts of nitrogen oxides present in combustion gases formed by the direct-fired heating systems found in traditional curing barns (Peele and Gentry (1999) "Formation of Tobacco-specific Nitrosamines in Flue-cured Tobacco," CORESTA Meeting, Agro-Phyto Groups, Suzhou, China). Retrofitting these curing barns with heat-exchangers virtually eliminated the mixing of combustion gases with the curing air and dramatically reduced the formation of TSNAs in tobaccos cured in this manner (Boyette and Hamm (2001) *Rec. Adv. Tob, Sci.* 27:17-22.). In contrast, in the air-cured Burley tobaccos, TSNA formation proceeds primarily through reaction of tobacco alkaloids with nitrite, a process catalyzed by leaf-borne microbes (Bush et al. (2001) *Rec. Adv. Tob. Sci,* 27:23-46). Thus far, attempts to reduce TSNAs through modification of curing conditions while maintaining acceptable quality standards have not proven to be successful for the air-cured tobaccos.

SUMMARY OF THE INVENTION

In an aspect, the present invention includes a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In another aspect, the present invention includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In a further aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In an aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions.

In an aspect, the present invention includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to-a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, and the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from burning the leaf as compared to burning a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions.

In a further aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718,

where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco.

In another aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco, and where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions.

In an aspect, the present invention includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present invention includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In an aspect, the present invention includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole and the regenerated plant has essentially all of the morphological and physiological characteristics of cultivar NCBEX1F when grown under the same environmental conditions.

In another aspect, the present invention includes a seed of tobacco cultivar NC EX90.

In an aspect, the present invention includes a tobacco plant, or part thereof, produced by growing a seed of tobacco cultivar NC EX90.

In a further aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NC EX90.

In another aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NC EX 90, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions.

In an aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NC EX90, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, and the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from burning the leaf as compared to burning a leaf from TN 90 LC or most other commercial burley tobacco cultivars when grown under similar conditions.

In a further aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NC EX90, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NC EX90, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco.

In another aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NC EX90, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions.

In an aspect, the present invention includes a part of a tobacco plant, produced by growing a seed of tobacco cultivar NC EX90, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In a further aspect, the present invention includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NC EX90, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In an aspect, the present invention includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NC EX90, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole, and the regenerated plant has essentially all of the morphological and physiological characteristics of cultivar NC EX90 when grown under the same environmental conditions.

In another aspect, the present invention includes a seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In an aspect, the present invention includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In another aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant produced by

growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In an aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions.

In another aspect, the present invention includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars when grown under similar conditions, and the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from burning the leaf as compared to burning a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions.

In a further aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco.

In another aspect, the present invention includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco, where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions.

In an aspect, the present invention includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present invention includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In an aspect, the present invention includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole, and the plant has essentially all of the morphological and physiological characteristics of cultivar NCBEX1MS when grown under the same environmental conditions.

In an aspect, the present invention includes an F_1 progeny plant of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In an aspect, the present invention includes an F_1 progeny plant of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In an aspect, the present invention includes an F_1 progeny plant of tobacco cultivar NC EX90.

In another aspect, the present invention includes an F_1 progeny plant of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the F_1 plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the plant of tobacco cultivar NCBEX1F is the female parent.

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention includes an F_1 progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited

with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a container of F_1 progeny seeds produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention includes an F_t progeny plant produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a harvested leaf of an F_1 progeny plant produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention further includes a harvested leaf of an F_1 progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a harvested leaf of an F_1 progeny plant, wherein the harvested leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from burning the leaf as compared to burning a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, where the plant is produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention includes a tobacco product prepared from an F_1 progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F_1 progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the present invention further includes a tobacco product prepared from an F_1 progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F_1 progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco.

In an aspect, the present invention further includes a tobacco product prepared from an F_1 progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F_1 progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions.

In an aspect, the present invention includes a progeny plant of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In an aspect, the present invention includes a progeny plant of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In an aspect, the present invention includes a progeny plant of tobacco cultivar NC EX90.

In another aspect, the present invention includes a progeny plant of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the progeny plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718.

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where the plant of tobacco cultivar NCBEX1F is the female parent.

In another aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention includes a progeny seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a container of progeny seeds produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention includes a progeny plant produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a harvested leaf of a progeny plant produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present invention further includes a harvested leaf of a progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention also includes a harvested leaf of a progeny plant, wherein the harvested leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from burning the leaf as compared to burning a leaf from TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, where the plant is produced by growing a seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present invention includes a tobacco product prepared from a progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the present invention further includes a tobacco product prepared from a progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco.

In an aspect, the present invention further includes a tobacco product prepared from a progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions.

In an aspect, the present invention includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is cytoplasmic male sterile, where the cytoplasmic male sterile plant is a plant of tobacco cultivar NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719.

In an aspect, the present invention includes a method of vegetatively propagating a plant of a tobacco cultivar comprising the steps of (a) culturing tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and

NC EX90 to obtain a proliferated shoot; and (b) rooting the proliferated shoots to obtain a rooted plantlet.

In an aspect, the present invention includes a method of vegetatively propagating a plant of a tobacco cultivar comprising the steps of (a) culturing tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90 to obtain a proliferated shoot; (b) rooting the proliferated shoots to obtain a rooted plantlet; and (c) growing a plant from the rooted plantlet.

In an aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F_2 progeny seed; (d) growing the F_2 progeny seed and selecting a backcross F_2 progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90, when grown under the same environmental conditions.

In an aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F_2 progeny seed; (d) growing the F_2 progeny seed and selecting a backcross F_2 progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 when grown under the same environmental conditions, where the trait is cytoplasmic male sterility (CMS).

In an aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F_2 progeny seed; (d) growing the F_2 progeny seed and selecting a backcross F_2 progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 when grown under the same environmental conditions, and the desired trait, where the trait is cytoplasmic male sterility (CMS) and the CMS trait is obtained from the cytoplasm of *Nicotiana suaveolens*.

In an aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F_2 progeny seed; (d) growing the F_2 progeny seed and selecting a backcross F_2 progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 when grown under the same environmental conditions, and the desired trait, where the trait is cytoplasmic male sterility (CMS) and the CMS trait is obtained from the cytoplasm of *Nicotiana suaveolens*, and where the second tobacco plant is TN 90 LC CMS.

In an aspect, the present invention includes a tobacco plant produced by a method comprising introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F_2 progeny seed; (d) growing the F_2 progeny seed and selecting a backcross F_2 progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of

NCBEX1F, NCBEX1MS, and NC EX90, when grown under the same environmental conditions.

In another aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a plant of another tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing; small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof, to produce an F_1 progeny seed; (b) growing the F_1 progeny seed into an F_1 progeny plant and selecting the F_1 progeny plant having the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross F2 progeny plant seed; (d) growing the backcross F2 progeny plant seed into a backcross F2 progeny plant and selecting a F2 backcross progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected fourth or higher backcross progeny plant that comprises the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90, when grown under the same environmental conditions.

In another aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a plant of another tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing; small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof, to produce an F_1 progeny seed; (b) growing the F_1 progeny seed into an F_1 progeny plant and selecting the F_1 progeny plant having the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross progeny plant seed; (d) growing the backcross progeny plant seed into a backcross progeny plant and selecting the backcross progeny plant comprising the desired trait; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected fourth or higher backcross progeny plant that comprises the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 when grown under the same environmental conditions.

In another aspect, the present invention includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90, with a plant of another tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing; small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof, to produce an F_1 progeny seed; (b) growing the F_1 progeny seed into an F_1 progeny plant and selecting the F_1 progeny plant having the desired trait; (c) crossing the selected F_1 progeny plant with a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 to produce a backcross progeny plant seed; (d) growing the backcross progeny plant seed into a backcross progeny plant and selecting the backcross progeny plant comprising the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90, when grown under the same environmental conditions; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected fourth or higher backcross progeny plant that comprises the desired trait and essentially all of the physiological and morphological characteristics of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90, when grown under the same environmental conditions, where the plant has the desired trait of disease resistance.

In another aspect, the present invention includes a method for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In an aspect, the present invention includes a method for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID

NO: 1 or SEQ ID NO: 2, where the second tobacco plant has the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In an aspect, the present invention includes a method for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, where the second tobacco plant does not have the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2 and the third tobacco plant is a tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In an aspect, the present invention includes for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, where the third tobacco plant is a tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

In an aspect, the present invention includes a method for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, where the first tobacco plant is a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90.

In an aspect, the present invention includes a method for producing a tobacco plant having decreased and more stable nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions comprising: identifying a first tobacco plant having the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2; crossing the first tobacco plant with a second tobacco plant and collecting an F_1 seed; selfing a plant grown from the F_1 seed or crossing a plant grown from the F_1 seed to a third tobacco plant to obtain a F_2 seed; and identifying a tobacco plant grown from the F_2 seed that is homozygous for the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, where the third tobacco plant is a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90.

In another aspect, the present invention includes a method of producing a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, NCBEX1MS, and NC EX90 having an additional desired trait comprising the steps of: (a) introducing a transgene conferring a desired trait into tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, NCBEX1MS, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719, and NC EX90.

In another aspect, the present invention includes a method of producing an herbicide resistant tobacco plant comprising transforming a tobacco plant, or part thereof, produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene where the transgene confers resistance to an herbicide. In some non-limiting examples, the herbicide is selected from the group consisting of imidazolinone, cyclohexanedione, sulfonylurea, glyphosate, glufosinate, phenoxy proprionic acid, L-phosphinothricin, triazine and benzonitrile.

In another aspect, the present invention includes an herbicide resistant tobacco plant produced by a method comprising transforming a tobacco plant, or part thereof, produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene where the transgene confers resistance to an herbicide selected from the group consisting of imidazolinone, cyclohexanedione, sulfonylurea, glyphosate, glufosinate, phenoxy proprionic acid, L-phosphinothricin, triazine and benzonitrile.

In another aspect, the present invention includes a method of producing a pest or insect resistant tobacco plant where the method comprises transforming a tobacco plant produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene that confers pest or insect resistance.

In a further aspect, the present invention includes a pest or insect resistant tobacco plant produced by a method comprising transforming a tobacco plant produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene that confers pest or insect resistance.

In a further aspect, the present invention includes a pest or insect resistant tobacco plant produced by a method comprising transforming a tobacco plant produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene that confers pest or insect resistance. In some aspects, the transgene encodes a *Bacillus thuringiensis* (BT) endotoxin.

In another aspect, the present invention includes a method of producing a disease resistant tobacco plant where the method comprises transforming a tobacco plant produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with

the ATCC under ATCC Accession No. PTA-11718, with a transgene that confers disease resistance.

In a further aspect, the present invention includes a disease resistant tobacco plant produced by a method comprising transforming a tobacco plant produced by growing a seed of tobacco cultivar NCBEX1F, a representative sample seed of the cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718, with a transgene that confers disease resistance.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 sets forth a cyp82e4 W329Stop nucleotide sequence.

SEQ ID NO: 2 sets forth a cyp82e5v2 W422Stop nucleotide sequence.

SEQ ID NO: 3 sets forth a cyp82e4 W329Stop amino acid sequence.

SEQ ID NO: 4 sets forth a cyp82e5v2 W422Stop amino acid sequence.

SEQ ID NO: 5 sets forth a CYP82E4 wild-type nucleotide sequence.

SEQ ID NO: 6 sets forth a CYP82E5v2 wild-type nucleotide sequence.

SEQ ID NO: 7 sets forth a CYP82E4 wild-type amino acid sequence.

SEQ ID NO: 8 sets forth a CYP82E5v2 wild-type amino acid sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts TN 90 LC certified plants and NC EX90 ("TN 90 RC") plants.

FIGS. **2**A and **2**B set forth the percentage of nicotine conversion for 50 plants of TN 90 LC and NCBEX1MS, respectively.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described more fully hereinafter with reference to the accompanying drawings and specification, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as an amount of a compound (e.g., an amount of nornicotine) and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, ±0.5%, or even ±0.1% of the specified amount.

NC EX90

The present invention includes tobacco cultivars, and parts thereof, from NC EX90. In another aspect, the present invention includes a tobacco plant, or part thereof, produced by growing the seed of NC EX90. A plant of the present invention can include a plant with essentially all of the morphological and physiological characteristics of cultivar NC EX90, when grown under the same environmental conditions.

While not being limited by process, NC EX90 is a result of the introduction of two mutated CYP82 genes in a Tennessee 90 Low Converter ("TN 90 LC") cultivar. The two genes are a mutated CYP82E4 gene recited as 325-6 #775 in Lewis et al. (Three nicotine demethylase genes mediate nornicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene, *Phytochemistry* 71 (2010) 1988-1998 (SEQ ID NO: 1, which sets forth a cyp82e4 W329Stop, hereby incorporated by reference in its entirety)), and a mutated CYP82E5v2 recited in Lewis et al. (supra) as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a cyp82e5v2 W422Stop, all references hereby incorporated by reference in their entirety). Both of these mutations result in truncated proteins. A cyp82e4 W329Stop and a cyp82e5v2 W422Stop are introduced from a e4e51e4e5 double mutant in a strong converter burley background, line DH98-325-6, as listed in Table 2 of Lewis et al. (supra) into a TN 90 LC background.

NC EX90 is generated by backcrossing with TN 90 LC five times as the recurrent parent and selfing twice. NC EX90 is homozygous for both a cyp82e4 W329Stop and a cyp82e5v2 W422Stop. Again, not limited by any particular scientific theory, a cyp82e4 W329Stop and a cyp82e5v2 W422Stop are recessive. A cyp82e4 W329Stop and a cyp82e5v2 W422Stop encode proteins with reduced or eliminated ability to convert nicotine or nornicotine. NC EX90 has a genetic background that is at least 95%, at least 97%, at least 98%, or at least 99% similar to TN 90 LC. NC EX90 exhibits low NNN and is not subject to conversion to high NNN's. (See, FIG. **1**)

NCBEX1MS

The present invention also provides tobacco cultivars, and parts thereof, from NCBEX1MS, where representative sample seeds of this cultivar have been deposited with the ATCC under ATCC Accession No. PTA-11719. The present invention also includes a tobacco plant, or part thereof, produced by growing a seed of NCBEX1MS. A plant of the present invention can include a plant with essentially all of the morphological and physiological characteristics of cultivar NCBEX1MS, when grown under the same environmental conditions. While not being limited by process, NCBEX1MS is a result of introducing the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations from NC EX90 BC_5F_1 into a TN 90 LC cytoplasmic male sterile ("CMS") plant by crossing NC EX90 BC_5F_1 as the male parent with TN 90 LC CMS to prepare CMS F_1 progeny plants.

The CMS F_1 progeny plants of the BC_5F_1×TN 90 LC CMS cross are male sterile. A plurality of BC_5F_1×TN 90 LC CMS F_1 plants (e.g., CMS F_1 progeny plants) are screened for the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations and crossed as the female parent to NC EX90 to prepare BC_7F_1 CMS progeny. BC_7F_1 CMS progeny homozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations are identified by genotyping and designated as NCBEX1MS. NCBEX1MS has a genetic background that is at least 95%, at

least 97%, at least 98%, or at least 99% similar to TN 90 LC. NCBEX1MS exhibits low NNN and is not subject to conversion to high NNN's.

NCBEX1F

The present invention also provides tobacco cultivars, and parts thereof, from NCBEX1F, representative sample seeds of this cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718. The present invention also includes a tobacco plant, or part thereof, produced by growing a seed of NCBEX1F. A plant of the present invention can further include a plant with essentially all of the morphological and physiological characteristics of cultivar NCBEX1F, when grown under the same environmental conditions. NCBEX1F is the result of seven backcrosses with TN 90 LC as the recurrent parent, followed by two rounds of selfing with selection for homozygosity for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations to yield BC_7F_3 plants. NCBEX1F has a genetic background that is at least 95%, at least 97%, at least 98%, or at least 99% similar to TN 90 LC. NCBEX1F exhibits low NNN and is not subject to conversion to high NNN's.

Other Plants

A progeny plant of the present application can be a plant of F_1, F_2, F_3, F_4 or later generation obtained by either crossing two parental plants or selfing one plant.

Under similar conditions as defined in the present application can be under similar environmental conditions or under similar laboratory conditions.

The present invention includes a tobacco seed produced by crossing two parent tobacco plants and harvesting the resultant tobacco seed, where at least one parent tobacco plant is NCBEX1F. In one aspect, the NCBEX1F is the male parent plant. In another aspect, the NCBEX1MS is the female parent plant. One aspect of the present invention provides tobacco plants that are homozygous at both a cyp82e4 and a cyp82e5v2 loci for SEQ ID NO: 1 and SEQ ID NO: 2, respectively, which share a genetic background that is greater than 75%, 80%, 85%, 90%, 95%, 98%, or 99% TN90 or TN90 LC. In one aspect, approximate or greater than 50%, 75%, or 100% of a progeny's genetics is provided by a plant of the present invention that is homozygous at both a cyp82e4 and a cyp82e5v2 loci for SEQ ID NO: 1 and SEQ ID NO: 2. In one aspect, a plant of the present invention has a genetic background that is at least 95%, at least 97%, at least 98%, or at least 99% similar to TN 90 LC. In another aspect, a plant of the present invention exhibits low NNN and is not subject to conversion to high NNN's. In one aspect, a plant of the present invention is the progeny plant of a female or male parent plant that is *Fusarium* wilt resistant.

In one aspect, a plant of the present invention is a medium-late maturing variety with moderately high yield potential. In another aspect, a plant of the present invention offers a broad range of important agronomic characteristics. In a further aspect, a plant of the present invention has one, two, three, four or more of the traits including moderate resistance to black shank, some tolerance to blue mold, black root rot resistance, and resistance to common virus diseases. In another aspect, a plant of the present invention has blue mold tolerance and level 4 resistance to both races of black shank and high root rot resistance. In one aspect, a plant of the present invention, such as NCBEX1F, NCBEX1MS, and NC EX90, lacks *Fusarium* wilt resistance. In another aspect, a plant of the present invention is *Fusarium* wilt resistant.

In an aspect, the plants of the present invention have reduced or eliminated ability to convert nicotine to nornicotine. In an aspect, the percentage nicotine conversion is less than about 75%, about 70%, about 60%, about 50%, or about 25% of that found in TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions. The nicotine conversion in plants of the present invention, including NCBEX1F, NCBEX1MS, and NC EX90, can be less than about 4%, about 3.5%, about 3%, about 2.5%, about 2%, about 1.5%, about 1%, about 3-1%, about 3-0.5%, or about 2-0.5%. In a preferred aspect, the percentage nicotine conversion is less than about 25%, about 10%, about 5%, or about 2% of that found in TN90 without a cyp82e4 W329Stop and a cyp82e5v2 W422Stop. In an aspect, the tobacco plants of the present invention have a nicotine conversion rate of about 3.5, about 3.25, about 3.0 or about 2.75% or less. In another aspect, the nicotine conversion rate of tobacco plants of the present invention can be about 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5% or less. In another aspect, the nicotine conversion rate of tobacco plants of the present invention can be about 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6% or less. In another aspect, the nicotine conversion rates can be from about 0.5 to 0.9%, 0.5 to 1.5%, 0.5 to 2.0%, 0.5 to 2.5%, 0.5 to 2.75%, and 0.5 to 3.0%. In another aspect, the nicotine conversion rates can be from about 1.0 to 1.5%, 1.0 to 1.75%, 1.0 to 2.0%, 1.0 to 2.5%, 1.0 to 2.75%, and 1.0 to 3.0%. In another aspect, the nicotine conversion rate in a plant of the present invention may be less than about 2.9, 2.75, 2.5, 2.25, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1 or 1.0%. In an aspect, the tobacco plants of the present invention have a nicotine conversion rate of 3.5, 3.25, 3.0 or 2.75% or less.

In another aspect, the tobacco plants of the present invention typically have a reduced amount of nornicotine of less than about 0.10% dry weight as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions. For example, the nornicotine content in such plants can be about 1.2, 1.0, 0.7, 0.5, 0.4, 0.2, 0.1, 0.09, 0.085, 0.08, 0.075, 0.07, 0.065, 0.06, 0.055, 0.05, 0.045, 0.04, 0.035, 0.025, 0.01, 0.009, 0.0075, 0.005, 0.0025, 0.001, 0.0009, 0.00075, 0.0005, 0.00025, 0.0001% dry weight, or undetectable. In another aspect, the nornicotine content can be less than about 1.2, 1.0, 0.9, 0.8, 0.7, 0.5, 0.4, 0.2, 0.1, 0.075, 0.05, 0.025, 0.01, 0.009, 0.0075, 0.005, 0.0025, 0.001, 0.0009, 0.00075, 0.0005, 0.00025, 0.0001% dry weight. In another aspect, the nornicotine content in such plants can be from about 1.2-1.0, 0.7-0.5, 0.4-0.2, 0.1-0.075, 0.05-0.025, 0.01-0.0075, 0.005-0.0025, 0.001-0.00075, 0.0005-0.00025, or 0.0005-0.0001% dry weight. In a plant of the present invention, the nornicotine is a relatively small percentage of total alkaloids in the plant compared to a commercial seedlot of TN90 LC. The nornicotine in a plant of the present invention may be 2-1%, less than 3%, about 2%, about 1.5%, about 1%, or 0.75% percentage of total alkaloids.

Tobacco products having a reduced amount of nitrosamine content and/or more stably low nicotine conversion as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions can be manufactured using tobacco plant material from plants and plant parts of the present invention. The tobacco product produced from a tobacco plant of this invention can have an amount of nornicotine of less than about 3 mg/g. For example, the nornicotine content in such a product can be about 3.0 mg/g, 2.5 mg/g, 2.0 mg/g, 1.5 mg/g, 1.0 mg/g, 750 μg/g, 500 pg/g, 250 pg/g, 100 pg/g, 75 pg/g, 50 pg/g, 25 pg/g, 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable. In some aspects, a tobacco product

produced from a tobacco plant of this invention can have a amount of NNN of less than about 10 pg/g. For example, the NNN content in such a product can be about 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable. The percentage of secondary alkaloids relative to total alkaloid content contained in a plant of the present invention may not be statistically different than from a commercial seedlot of TN90 LC.

Differences between two inbred tobacco varieties or two hybrid tobacco varieties can be evaluated using statistical approaches. Statistical analysis includes the calculation of mean values, determination of the statistical significance of the sources of variation, and the calculation of the appropriate variance components. Methods for determining statistical significance are known in the art. Statistical software is available, for example, the PROC GLM function of SAS. Significance is generally presented as a "p-value". A statistically significant p-value is less than 0.10. In a preferred aspect, the p-value is less than, or equal to, 0.05. In another aspect, the p-value is 0.04 or less, 0.03 or less, 0.02 or less. In yet another aspect, a statistically significant value is less than 0.01. In yet another aspect, it can be less than 0.009, less than 0.008, less than 0.007, less than 0.006, less than 0.005, less than 0.004, less than 0.003, less than 0.002, or less than 0.001.

Tobacco plants of the present invention that are homozygous for cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles have a reversion rate that is statistically significantly lower than corresponding control low-converter plants having wild type nicotine demethylase CYP82E4 and E5 genes. In addition, homozygous CYP82E4 and CYP82E5 double mutant tobacco plants have a percent conversion of nicotine to nornicotine of less than about 2.0%, e.g., undetectable to about 2.0%, about 1.0 to 2.0%, about 0.8 to 1.8%, about 0.8 to 2.0%, or about 1.0 to 2.0%.

Nicotine and nornicotine can be measured in ethylene-treated leaves using methods known in the art (e.g., gas chromatography). Percent nicotine demethylation in a sample is calculated by dividing the level of nornicotine by the combined level of nicotine and nornicotine as measured in the sample, and multiplying by 100. Percent nicotine demethylation in a sample from a plant of the present invention is 50, 40, 30, 20, 10 percent of a sample from an individual plant grown from a commercial seedlot of TN 90.

In an aspect, the tobacco plants of the present invention have a USDA quality index of about 73, about 72, about 71, about 70, about 69, about 68, about 67 or about 66. In an aspect, the tobacco plants of the present invention have a USDA quality index of about 65. In another aspect, the quality index may be at least about 55, 60, 62.5 or greater. In another aspect, tobacco plants of the present invention can have a quality index in the range of, about 60-65, about 60-70, about 62.5-65, about 62.5-70, or about 65-70, or any range therein.

A plant of the present invention, including NCBEX1MS, NCBEX1F, and NC EX90, can have any yield, including high (e.g., over 3000 lbs/A), moderately high (e.g., 2200-3000 lbs/A), and moderate (e.g., less than 2000 lbs/A) yield potential.

In another aspect, the present invention also provides for a plant grown from the seed of a NCBEX1F, NCBEX1MS, or NC EX90 plant in which alkaloids obtained from tobacco plants grown for the seed have decreased nornicotine and/or more stably low nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions, as well as plant parts and tissue cultures from such plants, representative sample seeds of these cultivars having been deposited with the ATCC, for example under ATCC Accession No. PTA-11718 for NCBEX1F and ATCC Accession No. PTA-11719 for NCBEX1MS.

An aspect of the present invention provides for parts of the cultivars NCBEX1F, NCBEX1MS, and NC EX90. For example, as used herein, a plant part includes but is not limited to leaves, pollen, embryos, cotyledons, hypocotyls, roots, root tips, anthers, flowers, ovules, shoots, stems, stalks, pith and capsules, petioles, pods, tissue culture comprising tissue, callus, cells or protoplasts of the cultivars NCBEX1F, NCBEX1MS, and/or NC EX90. In another aspect, the present invention provides for parts from hybrids having cultivars NCBEX1F, NCBEX1MS, and/or NC EX90 as parents or ancestors, and NCBEX1F, NCBEX1MS, and/or NC EX90 derived tobacco plants. In yet another aspect, the present invention provides for parts from genetically modified (e.g., by conventional breeding or genetic engineering techniques) forms of the foregoing plants and tissue culture.

Additional aspects of the present invention provide products comprising tobacco wherein the tobacco further comprises tobacco from the plants of the present invention, and parts thereof. Other aspects of the invention provide cured plant leaves and other plant parts from the plants of the present invention. Accordingly, in some aspects the cured plant parts include, but are not limited to, a leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod, petiole, capsules and the like, and combinations thereof.

Thus, in some aspects, the present invention provides a cured tobacco comprising the leaves of the tobacco plant designated NCBEX1F, a representative sample seed of said cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718. In another aspect the present invention provides a cured tobacco comprising the leaves of the tobacco plant designated NCBEX1MS, a representative sample seed of said cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719. In yet another aspect, the present invention provides a cured tobacco comprising the leaves of the tobacco plant designated NC EX90.

In an aspect, the present invention provides a cured tobacco comprising the stems of the tobacco plant designated NCBEX1F, a representative sample seed of said cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11718. In another aspect the present invention provides a cured tobacco comprising the stems of the tobacco plant designated NCBEX1MS, a representative sample seed of said cultivar having been deposited with the ATCC under ATCC Accession No. PTA-11719. In yet another aspect, the present invention provides a cured tobacco comprising the stems of the tobacco plant designated NC EX90.

In still other aspects, the present invention provides a cured tobacco comprising the leaves and stems of the tobacco plants designated NCBEX1F, NCBEX1MS, and/or NC EX90, representative sample seeds of these cultivars having been deposited with the ATCC under ATCC Accession No. PTA-11718 for NCBEX1F and ATCC Accession No. PTA-11719 for NCBEX1MS.

The present invention also provides a container of NCBEX1F, NCBEX1MS, or NC EX90 seeds or other seeds of the present invention (e.g., hybrids, inbreds, and the like) in which alkaloids obtained from tobacco plants grown from greater than 50% of the seeds have decreased nornicotine and/or more stably low nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions. In another aspect, alkaloids obtained from NCBEX1F, NCBEX1MS, or NC EX90 plants

or other plants of the present invention grown from greater than 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% of the seeds in the container have decreased nornicotine and/or more stably low nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown under similar conditions, representative sample seeds of cultivars NCBEX1F and NCBEX1MS having been deposited with the ATCC under ATCC Accession No. PTA-11718 for NCBEX1F and ATCC Accession No. PTA-11719 for NCBEX1MS.

A container of NCBEX1F, NCBEX1MS, or NC EX90 seeds or other seeds of the present invention may contain any number, weight or volume of seeds. For example, a container can contain at least, or greater than, about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000 or more seeds. Alternatively, the container can contain at least, or greater than, about 1 ounce, 5 ounces, 10 ounces, 1 pound, 2 pounds, 3 pounds, 4 pounds, 5 pounds or more seeds. Representative sample seeds of NCBEX1F and NCBEX1MS cultivars having been deposited with the ATCC under ATCC Accession Nos. PTA-11718 and PTA-11719, respectively.

Containers of NCBEX1F, NCBEX1MS, or NC EX90 seeds or other seeds of the present invention may be any container available in the art. By way of a non-limiting example, a container may be a box, a bag, a packet, a pouch, a tape roll, a pail, a foil, or a tube, representative sample seeds of these cultivars having been deposited with the ATCC. Representative sample seeds of NCBEX1F and NCBEX1MS cultivars have been deposited with the ATCC under ATCC Accession Nos. PTA-11718 and PTA-11719, respectively.

In another aspect, the present invention also provides a container of NCBEX1F, NCBEX1MS, or NC EX90 seeds in which greater than 50% of NCBEX1F, NCBEX1MS, or NC EX90 seeds or other seeds of the present invention have decreased nornicotine and/or more stably low nicotine conversion as compared to TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions. Representative sample seeds of NCBEX1F and NCBEX1MS cultivars have been deposited with the ATCC under ATCC Accession Nos. PTA-11718 and PTA-11719, respectively.

In one aspect, the present invention provides a seed of a NCBEX1F, NCBEX1MS, or NC EX90 plant or other plant of the present invention in which a plant grown from the seed is male sterile. Representative sample seeds of NCBEX1F and NCBEX1MS cultivars have been deposited with the ATCC under ATCC Accession Nos. PTA-11718 and PTA-11719, respectively.

Tobacco material, including without limitation any plant parts, for example leaves, midveins, and stalks, obtained from the tobacco cultivars, lines, varieties or hybrids of the present invention can be used to make tobacco products including, without limitation, cigarette products (e.g., cigarettes and bidi cigarettes), cigar products (e.g., cigar wrapping tobacco and cigarillos), pipe tobacco products, smokeless cigarette products, smokeless tobacco products (e.g., moist snuff, dry snuff, and chewing tobacco), films, chewables, tabs, shaped parts, gels, consumable units, insoluble matrices, hollow shapes and the like. See e.g., U.S. Patent Publication No. US 2006/0191548, which is herein incorporated by reference in its entirety.

Tobacco products derived from plants of the present invention also include cigarettes and other smoking articles, particularly those smoking articles including filter elements, wherein the rod of smokeable material includes cured tobacco within a tobacco blend. In an aspect, a tobacco product may be pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and cut tobacco.

In an aspect, the tobacco product of the present invention can be a blended tobacco product. In other aspects of the invention, the tobacco product of the present invention can be a reduced nicotine tobacco product. In still other aspects, the tobacco product of the present invention can be a blended tobacco product with reduced nicotine content. Thus, the tobacco product of the present invention can be a blended reduced nicotine tobacco product. Tobacco product material comprises a blend of tobacco materials from the present invention, wherein the blend comprises at least about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95 percent by weight of a cured tobacco, based on the dry weight of the tobacco material. US 2008/0245377 is herein incorporated by reference for blend mixtures in its entirety.

In an aspect, tobacco products having a reduced amount of nitrosamine content can be manufactured using tobacco plant material from plants and/or plant parts of the present invention. The tobacco product typically has a reduced amount of nornicotine of less than about 3 mg/g as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions. For example, the nornicotine content in such a product can be 3.0 mg/g, 2.5 mg/g, 2.0 mg/g, 1.5 mg/g, 1.0 mg/g, 750 μg/g, 500 pg/g, 250 pg/g, 100 pg/g, 75 pg/g, 50 pg/g, 25 pg/g, 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable. The tobacco product comprising tobacco material from a plant or plant part thereof of this invention typically has a reduced amount of NNN of less than about 10 pg/g as compared to a product prepared from TN 90 LC or most other commercial burley tobacco cultivars grown and processed under similar conditions. For example, the nornicotine content in such a product can be about 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable. The percentage of secondary alkaloids relative to total alkaloid content contained in a plant of the present invention may not be statistically different than from a commercial seedlot of TN90 LC.

A tobacco plant of the present invention designated NCBEX1F, NCBEX1MS, or NC EX90, carrying cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles can be used in a plant breeding program to create useful lines, cultivars, varieties, progeny, inbreds, and/or hybrids. Thus, in some aspects, an F_1, F_2, F_3, or later generation tobacco plant containing cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles is crossed with a second *Nicotiana* plant, and progeny of the cross are identified in which the cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles are present. It will be appreciated that the second *Nicotiana* plant will be TN90 or TN90 LC, optionally with an additional desirable trait. In some aspects the additional desirable trait can be herbicide resistance mentioned below. It will also be appreciated that the second TN90 or TN90 LC *Nicotiana* plant can contain cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles.

In still other aspects, methods of the present invention further include self-pollinating or pollinating a male sterile pollen acceptor with a pollen donor capable of being used in production of a progeny plant of the present invention, such as a male sterile hybrid of the present invention. Either the male sterile pollen acceptor plant or the pollen donor plant has at least one mutant allele, preferably two, at a nicotine demethylase locus, such as cyp82e4 W329Stop and cyp82e5v2 W422Stop. In an aspect, both alleles at each nicotine dem-

ethylase locus are mutant alleles, making the plant homozygous for cyp82e4 W329Stop and cyp82e5v2 W422Stop.

Breeding can be carried out via any known procedures. DNA fingerprinting, SNP or similar technologies may be used in a marker-assisted selection (MAS) breeding program to transfer or breed mutant alleles of a nicotine demethylase gene into other tobaccos. For example, a breeder can create segregating populations from hybridizations of a genotype containing cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles with an agronomically desirable genotype. Plants in the F_2 or backcross generations can be screened using a marker developed from cyp82e4 W329Stop or cyp82e5v2 W422Stop alleles or a fragment thereof, using one of the techniques known in the art or listed herein. Plants identified as possessing cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles can be backcrossed or self-pollinated to create a second population to be screened. Depending on the expected inheritance pattern or the MAS technology used, it may be necessary to self-pollinate the selected plants before each cycle of backcrossing to aid identification of the desired individual plants. Backcrossing or other breeding procedure can be repeated until the desired phenotype of the recurrent parent is recovered. A recurrent parent in the present invention can be NCBEX1F or NC EX90. Other breeding techniques can be found, for example, in Wernsman, E. A., and Rufty, R. C. 1987. Chapter Seventeen. Tobacco. Pages 669-698 In: Cultivar Development. Crop Species. W. H. Fehr (ed.), MacMillan Publishing Go., Inc., New York, N.Y., incorporated herein by reference in their entirety.

Nicotiana species which exhibit breeding compatibility with *Nicotiana tabacum* include *Nicotiana amplexicaulis*, PI 271989; *Nicotiana benthamiana* PI 555478; *Nicotiana bigelovii* PI 555485; *Nicotiana debneyi; Nicotiana excelsior* PI 224063; *Nicotiana glutinosa* PI 555507; *Nicotiana goodspeedii* PI 241012; *Nicotiana gossei* PI 230953; *Nicotiana hesperis* PI 271991; *Nicotiana knightiana* PI 555527; *Nicotiana maritima* PI 555535; *Nicotiana megalosiphon PI* 555536; *Nicotiana nudicaulis* PI 555540; *Nicotiana paniculata* PI 555545; *Nicotiana plumbaginifolia* PI 555548; *Nicotiana repanda* PI 555552; *Nicotiana rustica; Nicotiana suaveolens* PI 230960; *Nicotiana sylvestris* PI 555569; *Nicotiana tomentosa* PI 266379; *Nicotiana tomentosiformis*; and *Nicotiana trigonophylla* PI 555572. See also, Compendium of Tobacco Diseases published by American Phytopathology Society, or The Genus *Nicotiana* Illustrated, published by Japan Tobacco Inc, hereby incorporated by reference in their entirety.

The result of a plant breeding program using the tobacco plants described herein includes useful lines, cultivars, varieties, progeny, inbreds, and hybrids. As used herein, the term 'variety' refers to a population of plants that share constant characteristics which separate them from other plants of the same species. A variety is often, although not always, sold commercially. While possessing one or more distinctive traits, a variety is further characterized by a very small overall variation between individuals within that variety. A 'pure line' variety may be created by several generations of self-pollination and selection, or vegetative propagation from a single parent using tissue or cell culture techniques. A variety can be essentially derived from another line or variety. As defined by the International Convention for the Protection of New Varieties of Plants (Dec. 2, 1961, as revised at Geneva on Nov. 10, 1972, on Oct. 23, 1978, and on Mar. 19, 1991), a variety is 'essentially derived' from an initial variety if: a) it is predominantly derived from the initial variety, or from a variety that is predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety; b) it is clearly distinguishable from the initial variety; and c) except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety. Essentially derived varieties can be obtained, for example, by the selection of a natural or induced mutant, a somaclonal variant, a variant individual from plants of the initial variety, backcrossing, or transformation. A 'line' as distinguished from a variety most often denotes a group of plants used non-commercially, for example in plant research. A line typically displays little overall variation between individuals for one or more traits of interest, although there may be some variation between individuals for other traits.

Hybrid tobacco varieties can be produced by preventing self-pollination of female parent plants (i.e., seed parents) of a first variety, permitting pollen from male parent plants of a second variety to fertilize the female parent plants, and allowing F_1 hybrid seeds to form on the female plants. Self-pollination of female plants can be prevented by emasculating the flowers at an early stage of flower development. Alternatively, pollen formation can be prevented on the female parent plants using a form of male sterility. For example, male sterility can be produced by cytoplasmic male sterility (CMS), or transgenic male sterility wherein a transgene inhibits microsporogenesis and/or pollen formation, or self-incompatibility. Female parent plants containing CMS are particularly useful. In aspects in which the female parent plants are CMS, pollen may be harvested from male fertile plants and applied manually to the stigmas of CMS female parent plants, and the resulting F_1 seed harvested.

Plants can be used to form single-cross tobacco F_1 hybrids. In such an aspect, the plants of the parent varieties can be grown as substantially homogeneous adjoining populations to facilitate natural cross-pollination from the male parent plants to the female parent plants. The F_1 seed formed on the female parent plants is selectively harvested by conventional means. One also can grow the two parent plant varieties in bulk and harvest a blend of F_1 hybrid seed formed on the female parent and seed formed upon the male parent as the result of self-pollination. Alternatively, three-way crosses can be carried out wherein a single-cross F_1 hybrid is used as a female parent and is crossed with a different male parent. As another alternative, double-cross hybrids can be created wherein the F_1 progeny of two different single-crosses are themselves crossed. Self-incompatibility can be used to particular advantage to prevent self-pollination of female parents when forming a double-cross hybrid.

In some aspects, successful crosses yield F_1 plants that are fertile, have cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles, and can be backcrossed with one of the parents, such as NCBEX1F or NC EX90 if desired. In some aspects, a plant population in the F_2 generation is screened for cyp82e4 W329Stop and cyp82e5v2 W422Stop alleles. Selected plants can be crossed with one of the parents and the first backcross (BC1) generation plants are self-pollinated to produce a BC1 F_2 population that is again screened for variant nicotine demethylase gene expression (e.g., the null version of the nicotine demethylase gene). The process of backcrossing, self-pollination, and screening is repeated, for example, at least 4 times, until the final screening produces a plant that is fertile and reasonably similar to the recurrent parent. This plant, if desired, is self-pollinated and the progeny are subsequently screened again to confirm that the plant exhibits the same low and stable nicotine conversion phenotype as NCBEX1F. Breeder's seed of the selected plant is produced using stan-

dard methods including, for example, field testing, confirmation of the null condition for nicotine demethylase, chemical analyses of cured leaf to determine the level of alkaloids and/or chemical analyses of cured leaf to determine the ratio of nornicotine to nicotine+nornicotine.

In one aspect, a F_1 progeny is the result of a cross between NCBEX1F and NCBEX1MS to generate F_1 progeny that are male sterile. Male sterile tobacco plants may be produced by any method known in the art. Methods of producing male sterile tobacco are described in Wernsman, E. A., and Rufty, R. C. 1987. Chapter Seventeen. Tobacco. Pages 669-698 In: Cultivar Development. Crop Species. W. H. Fehr (ed.), MacMillan Publishing Go., Inc., New York, N.Y. 761 pp.

The present invention further provides methods of producing a tobacco plant by crossing one of cultivars NCBEX1F, NCBEX1MS, and/or NC EX90 with itself or a different tobacco line. The invention further relates to methods for producing other tobacco cultivars or breeding lines derived from cultivars NCBEX1F, NCBEX1MS, and/or NC EX90 by crossing a plant of a cultivar of NCBEX1F, NCBEX1MS, and/or NC EX90 with a second tobacco plant and growing the progeny seed to yield a NCBEX1F-, NCBEX1MS-, or NC EX90-derived tobacco plant. An additional aspect of the present invention provides a method for producing a tobacco plant that contains in its genetic material one or more transgenes, comprising crossing cultivars of the present invention with a second tobacco plant containing one or more transgenes to produce progeny that comprise the transgene(s) optionally operably linked to one or more regulatory elements. In one aspect, the second cultivar may be a plant derived from cultivars NCBEX1F, NCBEX1MS, and/or NC EX90 transformed with one or more transgenes.

The invention further provides for the vegetative propagation of a plant of cultivars NCBEX1F, NCBEX1MS, and/or NC EX90, hybrids and progeny thereof. In one aspect, the invention provides for a method of vegetatively propagating a plant of a tobacco cultivar comprising cultivating tissue capable of being propagated from a plant of a plant of cultivars NCBEX1F, NCBEX1MS, and/or NC EX90, to obtain a proliferated shoot and rooting the proliferated shoots to obtain a rooted plantlet. In another aspect, the plant tissue may be from an F_1 hybrid of a plant of cultivars NCBEX1F, NCBEX1MS, and/or NC EX90. In an aspect, the plant tissue may be from an F_2, F_3, F_4 or later progeny plant obtained by breeding a plant of cultivars NCBEX1F, NCBEX1MS, and/or NC EX90.

A plant comprising a mutation in a nicotine demethylase gene can be identified by selecting or screening the mutagenized plant material, or progeny thereof. Such screening and selection methodologies are known to those having ordinary skill in the art. Examples of screening and selection methodologies include, but are not limited to, Southern analysis, PCR amplification for detection of a polynucleotide, Northern blots, RNase protection, primer-extension, RT-PCR amplification for detecting RNA transcripts, enzymatic assays for detecting enzyme or ribozyme activity of polypeptides and polynucleotides, and protein gel electrophoresis, Western blots, immunoprecipitation, and enzyme-linked immunoassays to detect polypeptides. Other techniques such as in situ hybridization, enzyme staining, and immunostaining also can be used to detect the presence or expression of polypeptides and/or polynucleotides. Methods for performing all of the referenced techniques are known.

It is understood that a tobacco plant of the present invention, including NCBEX1F, NCBEX1MS and/or NC EX90, can be transformed by a genetic construct or transgene using a technique known in the art. Without limitation, an example of a desired trait is herbicide resistance, pest resistance, disease resistance; high yield; high grade index; curability; curing quality; mechanical harvestability; holding ability; leaf quality; height, plant maturation (e.g., early maturing, early to medium maturing, medium maturing, medium to late maturing, or late maturing); stalk size (e.g., a small, medium, or a large stalk); or leaf number per plant (e.g., a small (e.g., 5-10 leaves), medium (e.g., 11-15 leaves), or large (e.g., 16-21) number of leaves), or any combination thereof. Any plant of the present invention can be used as a basis for tissue culture, regenerated, transformed, or a combination of any of these. In an aspect, a plant of the present invention derived by tissue culture, transformation, or both has essentially all of the morphological and physiological characteristics of cultivar NCBEX1F, NCBEX1MS or NC EX90 under similar conditions.

Having now generally described the invention, the same will be more readily understood through reference to the following examples that are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLES

Example 1

Breeding of Homozygous cyp82e4 W329Stop and cyp82e5v2 W422Stop Mutant Plants into the TN 90 LC Background

NC EX90 is a backcross-derived version of burley tobacco cultivar TN 90 LC carrying introduced deleterious mutations in cyp82e4 and cyp82e5v2, two genes previously documented to encode for nicotine demethylase enzymes (Lewis et al., supra). To prepare NC EX90 an individual plant grown from a commercial seedlot of TN 90 LC is selected and initially crossed with plant GH08B-14 in a greenhouse. GH08B-14 is a burley tobacco cultivar heterozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations described by Lewis et al. (2010). A plurality of F_1 plants are screened for the presence of the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations. Individual F_1 plants are selected and backcrossed to TN 90 LC in a greenhouse to produce BC_1F_1 progeny. A plurality of BC_1F_1 progeny are screened and an individual plant heterozygous for the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations is identified. The heterozygous selected BC_1F_1 plant is backcrossed to TN 90 LC in a greenhouse to produce BC_2F_1 seed. A plurality of BC_2F_1 plants are screened for the presence of the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations to identify a heterozygous progeny plant for a subsequent round of backcross breeding. Using this backcross procedure, individual heterozygous plants having the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations are identified in the BC_3F_1, BC_4F_1, and BC_5F_1 progeny.

To produce plants homozygous for the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations, BC_5F_1 progeny plants are screened for the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations to identify heterozygous plants. Individual plants heterozygous for the cyp82e4 W329Stop and cyp82e5v2 W422Stop mutations are self-pollinated to produce BC_5F_2 seed. A plurality of BC_5F_2 progeny are genotyped to identify individuals homozygous for both of the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations. Four individual BC_5F_2 progeny plants are self-pollinated to produce four BC_5F_3 progeny lines. BC_5F_3 are homozygous for the CYP82E4 W329Stop and CYP82E5v2 W422Stop mutations.

Identification of BC_5F_3 Progeny Lines with Desirable Traits

Plants from the four BC_5F_3 progeny lines are grown in a randomized complete block design with three replications for evaluation of cured leaf chemistry, yield, and physical quality

at three North Carolina field research locations during 2010 (Waynesville, Laurel Springs, and Reidsville). Each replicated block is a 20-plant plot. Plants are stalk cut at maturity, air cured and evaluated by a former USDA tobacco grader. Plot weights are used to determine per acre yields. Fifty gram composite leaf samples are collected from each plot and analyzed for percent nicotine, nornicotine, anatabine, and anabasine by gas chromatography. BC_5F_3 progeny line #3 is identified with superior yield, high grade and desirable alkaloid content and designated 'NC EX90.'

Analysis of NC EX90

Results of gas chromatography alkaloid analysis are presented in Table 1.

Analysis with single degree of freedom CONTRAST statements are performed using the PROC GLM function of SAS. This line exhibits significantly lower levels of nornicotine ($P<0.0001$) and nicotine conversion ($P=0.0002$) as compared to a commercial seedlot of TN 90 LC (Table 1). The ratio of secondary alkaloids to total alkaloids are significantly lower for NC EX90 relative to TN 90 LC ($P=0.0071$). The two lines are not significantly different for nicotine, anatabine, anabasine, yield, or cured leaf quality.

TABLE 1

Comparisons between TN 90 LC and NC EX90 (TN 90 LC + e4e4 + e5e5) for alkaloid determinations, yield, and cured leaf quality.

Entry	Nicotine (% dry weight)	Nornicotine (% dry weight)	Anatabine (% dry weight)	Anabasine (% dry weight)	Total Alkaloids (% dry weight)	Nicotine Conversion (%)	Ratio Secondary Alkaloids: Total Alkaloids	Yield (lbs/A)	Quality Index
TN 90 LC	3.579	0.158	0.216	0.022	3.980	3.918	0.096	2403.3	62.59
NC EX90	3.571	0.076	0.221	0.022	3.890	2.043	0.081	2454.5	65.70
P-value[a]	0.9622	<0.0001	0.7560	0.7059	0.6620	0.0002	0.0071	0.8856	0.5514

[a]P-values were obtained from single degree of freedom CONTRAST statements executed by PROC GLM of SAS.

Means are from three 2010 North Carolina environments. The experimental design at each location was a randomized complete block design with three replications.

Example 2

Preparation of Inbred Line NCBEX11

To prepare inbred line NCBEX1F, two additional rounds of backcrossing of individual BC_5F_1 progeny plants prepared in Example 1 above are performed. A plurality of BC_5F_1 plants are screened for the presence of the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations to identify a heterozygous progeny plant for a subsequent round of backcross breeding. Using this backcross procedure, individual heterozygous plants having the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations are identified in the BC_6F_1 and BC_7F_1 progeny.

To produce plants homozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations, BC_7F_1 progeny plants are screened for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations to identify heterozygous plants. Individual plants heterozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations are self-pollinated to produce BC_7F_2 seed. A plurality of BC_7F_2 progeny are genotyped to identify individuals homozygous for both of the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations. Individual BC_7F_2 progeny plants are self-pollinated to produce individual BC_7F_3 progeny lines. The NCBEX1F progeny lines are homozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations.

Example 3

Preparation of Cytoplasmic Male Sterile Lines

To prepare a cytoplasmic male sterile (CMS) line, a BC_5F_1 progeny plant prepared as described in Example 1 above that is heterozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations is selected and crossed as the pollen parent to a proprietary CMS sterile line of the North Carolina State University designated TN 90 LC CMS. TN 90 LC CMS is prepared by crossing a TN 90 LC plant with a plant of tobacco species *Nicotiana suaveolens*. The F_1 progeny plant is backcrossed nine times to TN 90 LC to produce BC_9F_1 plants and designated TN 90 LC CMS. The TN 90 LC CMS line is maintained by backcrossing to TN 90 LC.

The F_1 progeny plants of the BC_5F_1×TN 90 LC CMS are male sterile. A plurality of BC_5F_1×TN 90 CMS F_1 plants (e.g., the F_1 progeny plants) are screened for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations and crossed as the female parent to TN 90 LC to prepare BC_7F_1 CMS progeny. BC_7F_1 CMS progeny heterozygous for the cyp82e4 W329Stop and the cyp82e5v2 W422Stop mutations are identified by genotyping. Two rounds of crossing to NC EX90 are performed to prepare BC_7F_3 CMS plants and designated as line NCBEX1MS. To maintain the line, plants of line NCBEX1MS plants are pollinated with a NCBEX1F plant.

Example 4

Demonstration of Trait Stability in Nicotine Conversion for NCBEX1Ms Relative to TN 90LC

Leaves from 50 plants for NCBEX1MS and TN90LC are collected using the "LC" method as disclosed in Jack et al. Implications of reducing nornicotine accumulation in burley tobacco: Appendix A—The LC Protocol. *Rec. Adv. Tob. Sci.* 2007, 33, 58-79. Leaves are air cured and analyzed for alkaloid profiles using gas chromatography. See FIGS. **2**A and **2**B and Table 2.

Analysis with single degree of freedom CONTRAST statements are performed using the PROC GLM function of SAS. This line exhibits significantly lower levels of nornicotine ($P<0.05$) and nicotine conversion ($P<0.05$) as compared to a commercial seedlot of TN 90 LC. Rates of nicotine conversion and nornicotine levels are significantly reduced while yield and quality of NCBEX1MS lines is not significantly different from TN 90 LC (differences do not exceed the LSD at the 0.05 level of significance).

TABLE 2

Comparison of NCBEX1MS relative to TN 90 LC and standard checks for yield, quality, and cured leaf chemistry during the 2011 growing season.

Genotype	Yield (lbs/A)	Grade Index	Nicotine (%)	Nornicotine (%)	Anabasine (%)	Anatabine (%)	Nicotine Conversion (%)
VA509	2959.3	69.3	3.405	0.155	0.014	0.151	4.297
Ky 14 LC	3126.2	71.9	3.644	0.086	0.016	0.187	2.256
TN 90 LC (certified)	3139.7	70.7	3.845	0.159	0.016	0.220	3.932
NCBEX1MS	3142.9	72.2	3.955	0.080	0.016	0.200	1.971
LSD (0.05)	473.5	8.4	0.570	0.053	0.004	0.054	1.085

Means are averages over three 2011 field environments. The experimental design was a randomized complete block design with four replications at each environment.

DEPOSIT INFORMATION

A deposit of at least 2500 seeds of the proprietary inbred plant lines disclosed above and recited in the appended claims have been made with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The date of deposit for NCBEX1F and NCBEX1MS was Sep. 6, 2011 and Sep. 6, 2011, respectively. The deposit of 2500 seeds for each variety was taken from the same deposit maintained since prior to the filing date of this application. Upon issuance of a patent, all restrictions upon the deposit will be removed, and the deposit is intended to meet all of the requirements of 37 C.F.R. §1.801-1.809. The ATCC accession numbers for inbred lines NCBEX1F and NCBEX1MS are PTA-11718, PTA-11719, respectively. These deposits will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced as necessary during that period. Applicants do not waive any infringement of their rights granted under this patent or under the Plant Variety Protection Act (7 U.S.C. 2321 et seq.).

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ctttgttagt accacacgaa aatgtagaag attgtgttgt tagtggatat cacattccta 1260

aagggacaag attattcgca aacgtcatga aactgcaacg tgatcctaaa ctctggtctg 1320

atcctgatac tttcgatcca gagagattca ttgctactga tattgacttt cgtggtcagt 1380

actataagta tatcccgttt ggttctggaa gacgatcttg tccagggatg acttatgcat 1440

tgcaagtgga acacttaaca atggcacatt tgatccaagg tttcaattac agaactccaa 1500

atgacgagcc cttggatatg aaggaaggtg caggcataac tatacgtaag gtaaatcctg 1560

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tcttggttga tcattgtata atactcctaa atggatattc atttacctttt tatcaattaa 1680

ttgtcagtac gagtttttct aatttggtac atttgtaata ataagtaaag aataattgtg 1740

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<210> SEQ ID NO 6
<211> LENGTH: 1554

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<212> TYPE: DNA
<213> ORGANISM: Nicotiana tabacum

<400> SEQUENCE: 6

atggtttttc cggtagaagc cattgtagga ctagtaaccc ttacacttct cttctacttc 60

ctatggccca aaaaatttca aataccttca aaaccattac caccgaaaat tcccggaggg 120

tggccggtaa tcggccatct tttctacttc gatgatgacg gcgacgaccg tccattagct 180

cgaaaactcg gagacttagc tgacaaatac ggcccggttt tcactttccg gctaggcctt 240

ccgcttgtgt tagttgtaag cagttacgaa gctgtaaaag actgcttctc tacaaatgac 300

gccatttttct ccaatcgtcc agcttttctt tacggtgaat accttggcta cagtaatgcc 360

atgctatttt tgacaaaata cggaccttat tggcgaaaaa atagaaaatt agtcattcag 420

gaagttctct ctgctagtcg tctcgaaaaa ttgaagcacg tgagatttgg taaaattcaa 480

acgagcatta agagtttata cactcgaatt gatggaaatt cgagtacgat aaatctaact 540

gattggttag aagaattgaa ttttggtctg atcgtgaaaa tgatcgctgg gaaaaattat 600

gaatccggta aaggagatga acaagtggag agatttagga aagcgtttaa ggattttata 660

attttatcaa tggagtttgt gttatgggat gcttttccaa ttccattgtt caaatgggtg 720

gattttcaag gccatgttaa ggccatgaaa aggacattta aggatataga ttctgttttt 780

cagaattggt tagaggaaca tgtcaagaaa agagaaaaaa tggaggttaa tgcacaaggg 840

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ggttactctc gtgatactgt cataaaagca acagtgttta gtttggtctt ggatgctgcg 960

gacacagttg ctcttcacat gaattgggga atggcattac tgataaacaa tcaacatgcc 1020

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agtgatatta aggatttggt ctacctccaa gctattgtta aagaagtgtt acgattatat 1140

ccaccaggac ctttattagt acctcatgaa aatgtagagg attgtgttgt tagtggatat 1200

cacattccta aagggactag actattcgcg aacgttatga aattgcagcg cgatcctaaa 1260

ctctggtcaa atcctgataa gtttgatcca gagagattct tcgctgatga tattgactac 1320

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<211> LENGTH: 517
<212> TYPE: PRT
<213> ORGANISM: Nicotiana tabacum

<400> SEQUENCE: 7

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Leu Phe Phe Phe Leu Trp Thr Lys Lys Ser Gln Lys Pro Ser Lys Pro
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Leu Pro Pro Lys Ile Pro Gly Gly Trp Pro Val Ile Gly His Leu Phe
35 40 45

His Phe Asn Asp Asp Gly Asp Asp Arg Pro Leu Ala Arg Lys Leu Gly
50 55 60

Asp Leu Ala Asp Lys Tyr Gly Pro Val Phe Thr Phe Arg Leu Gly Leu
65 70 75 80

-continued

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Pro Leu Val Leu Val Val Ser Ser Tyr Glu Ala Val Lys Asp Cys Phe
                85                  90                  95

Ser Thr Asn Asp Ala Ile Phe Ser Asn Arg Pro Ala Phe Leu Tyr Gly
            100                 105                 110

Asp Tyr Leu Gly Tyr Asn Asn Ala Met Leu Phe Leu Ala Asn Tyr Gly
        115                 120                 125

Pro Tyr Trp Arg Lys Asn Arg Lys Leu Val Ile Gln Glu Val Leu Ser
    130                 135                 140

Ala Ser Arg Leu Glu Lys Phe Lys His Val Arg Phe Ala Arg Ile Gln
145                 150                 155                 160

Ala Ser Ile Lys Asn Leu Tyr Thr Arg Ile Asp Gly Asn Ser Ser Thr
                165                 170                 175

Ile Asn Leu Thr Asp Trp Leu Glu Glu Leu Asn Phe Gly Leu Ile Val
            180                 185                 190

Lys Met Ile Ala Gly Lys Asn Tyr Glu Ser Gly Lys Gly Asp Glu Gln
        195                 200                 205

Val Glu Arg Phe Lys Lys Ala Phe Lys Asp Phe Met Ile Leu Ser Met
    210                 215                 220

Glu Phe Val Leu Trp Asp Ala Phe Pro Ile Pro Leu Phe Lys Trp Val
225                 230                 235                 240

Asp Phe Gln Gly His Val Lys Ala Met Lys Arg Thr Phe Lys Asp Ile
                245                 250                 255

Asp Ser Val Phe Gln Asn Trp Leu Glu Glu His Ile Asn Lys Arg Glu
            260                 265                 270

Lys Met Glu Val Asn Ala Glu Gly Asn Glu Gln Asp Phe Ile Asp Val
        275                 280                 285

Val Leu Ser Lys Met Ser Asn Glu Tyr Leu Gly Glu Gly Tyr Ser Arg
    290                 295                 300

Asp Thr Val Ile Lys Ala Thr Val Phe Ser Leu Val Leu Asp Ala Ala
305                 310                 315                 320

Asp Thr Val Ala Leu His Ile Asn Trp Gly Met Ala Leu Leu Ile Asn
                325                 330                 335

Asn Gln Lys Ala Leu Thr Lys Ala Gln Glu Glu Ile Asp Thr Lys Val
            340                 345                 350

Gly Lys Asp Arg Trp Val Glu Glu Ser Asp Ile Lys Asp Leu Val Tyr
        355                 360                 365

Leu Gln Ala Ile Val Lys Glu Val Leu Arg Leu Tyr Pro Pro Gly Pro
    370                 375                 380

Leu Leu Val Pro His Glu Asn Val Glu Asp Cys Val Val Ser Gly Tyr
385                 390                 395                 400

His Ile Pro Lys Gly Thr Arg Leu Phe Ala Asn Val Met Lys Leu Gln
                405                 410                 415

Arg Asp Pro Lys Leu Trp Ser Asp Pro Asp Thr Phe Asp Pro Glu Arg
            420                 425                 430

Phe Ile Ala Thr Asp Ile Asp Phe Arg Gly Gln Tyr Tyr Lys Tyr Ile
        435                 440                 445

Pro Phe Gly Ser Gly Arg Arg Ser Cys Pro Gly Met Thr Tyr Ala Leu
    450                 455                 460

Gln Val Glu His Leu Thr Met Ala His Leu Ile Gln Gly Phe Asn Tyr
465                 470                 475                 480

Arg Thr Pro Asn Asp Glu Pro Leu Asp Met Lys Glu Gly Ala Gly Ile
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Thr Ile Arg Lys Val Asn Pro Val Glu Leu Ile Ile Ala Pro Arg Leu
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Ala Pro Glu Leu Tyr
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<210> SEQ ID NO 8
<211> LENGTH: 517
<212> TYPE: PRT
<213> ORGANISM: Nicotiana tabacum

<400> SEQUENCE: 8

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            20                  25                  30

Leu Pro Pro Lys Ile Pro Gly Gly Trp Pro Val Ile Gly His Leu Phe
        35                  40                  45

Tyr Phe Asp Asp Asp Gly Asp Asp Arg Pro Leu Ala Arg Lys Leu Gly
    50                  55                  60

Asp Leu Ala Asp Lys Tyr Gly Pro Val Phe Thr Phe Arg Leu Gly Leu
65                  70                  75                  80

Pro Leu Val Leu Val Val Ser Ser Tyr Glu Ala Val Lys Asp Cys Phe
                85                  90                  95

Ser Thr Asn Asp Ala Ile Phe Ser Asn Arg Pro Ala Phe Leu Tyr Gly
            100                 105                 110

Glu Tyr Leu Gly Tyr Ser Asn Ala Met Leu Phe Leu Thr Lys Tyr Gly
        115                 120                 125

Pro Tyr Trp Arg Lys Asn Arg Lys Leu Val Ile Gln Glu Val Leu Ser
    130                 135                 140

Ala Ser Arg Leu Glu Lys Leu Lys His Val Arg Phe Gly Lys Ile Gln
145                 150                 155                 160

Thr Ser Ile Lys Ser Leu Tyr Thr Arg Ile Asp Gly Asn Ser Ser Thr
                165                 170                 175

Ile Asn Leu Thr Asp Trp Leu Glu Glu Leu Asn Phe Gly Leu Ile Val
            180                 185                 190

Lys Met Ile Ala Gly Lys Asn Tyr Glu Ser Gly Lys Gly Asp Glu Gln
        195                 200                 205

Val Glu Arg Phe Arg Lys Ala Phe Lys Asp Phe Ile Ile Leu Ser Met
    210                 215                 220

Glu Phe Val Leu Trp Asp Ala Phe Pro Ile Pro Leu Phe Lys Trp Val
225                 230                 235                 240

Asp Phe Gln Gly His Val Lys Ala Met Lys Arg Thr Phe Lys Asp Ile
                245                 250                 255

Asp Ser Val Phe Gln Asn Trp Leu Glu Glu His Val Lys Lys Arg Glu
            260                 265                 270

Lys Met Glu Val Asn Ala Gln Gly Asn Glu Gln Asp Phe Ile Asp Val
        275                 280                 285

Val Leu Ser Lys Met Ser Asn Glu Tyr Leu Asp Glu Gly Tyr Ser Arg
    290                 295                 300

Asp Thr Val Ile Lys Ala Thr Val Phe Ser Leu Val Leu Asp Ala Ala
305                 310                 315                 320

Asp Thr Val Ala Leu His Met Asn Trp Gly Met Ala Leu Leu Ile Asn
                325                 330                 335

Asn Gln His Ala Leu Lys Lys Ala Gln Glu Glu Ile Asp Lys Lys Val
            340                 345                 350
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-continued

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Gly Lys Glu Arg Trp Val Glu Glu Ser Asp Ile Lys Asp Leu Val Tyr
        355                 360                 365

Leu Gln Ala Ile Val Lys Glu Val Leu Arg Leu Tyr Pro Pro Gly Pro
    370                 375                 380

Leu Leu Val Pro His Glu Asn Val Glu Asp Cys Val Val Ser Gly Tyr
385                 390                 395                 400

His Ile Pro Lys Gly Thr Arg Leu Phe Ala Asn Val Met Lys Leu Gln
                405                 410                 415

Arg Asp Pro Lys Leu Trp Ser Asn Pro Asp Lys Phe Asp Pro Glu Arg
            420                 425                 430

Phe Phe Ala Asp Asp Ile Asp Tyr Arg Gly Gln His Tyr Glu Phe Ile
        435                 440                 445

Pro Phe Gly Ser Gly Arg Arg Ser Cys Pro Gly Met Thr Tyr Ala Leu
    450                 455                 460

Gln Ala Glu His Leu Thr Ile Ala His Leu Ile Gln Gly Phe Asn Tyr
465                 470                 475                 480

Lys Thr Pro Asn Asp Glu Pro Leu Asp Met Lys Glu Gly Ala Gly Leu
                485                 490                 495

Thr Ile Arg Lys Val Asn Pro Val Glu Val Thr Ile Thr Ala Arg Leu
            500                 505                 510

Ala Pro Glu Leu Tyr
        515
```

What is claimed is:

1. A seed of tobacco cultivar NCBEX1F, a tobacco cultivar essentially derived from said tobacco cultivar NCBEX1F, or a tobacco hybrid produced from said tobacco cultivar NCBEX1F, wherein said seed comprises SEQ ID NO: 1 and SEQ ID NO: 2, a representative sample seed of said cultivar NCBEX1F is deposited with the ATCC under ATCC Accession No. PTA-11718, wherein said tobacco cultivar NCBEX1F, said tobacco cultivar essentially derived from said tobacco cultivar NCBEX1F, and said tobacco hybrid produced from said tobacco cultivar NCBEX1F comprises a percent nicotine conversion less than about 2.5%.

2. A tobacco plant, or a part thereof, produced by growing the seed of claim **1**, wherein said part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

3. A harvested leaf of the tobacco plant of claim **2**.

4. The harvested leaf of claim **3**, wherein said leaf has a reduced amount of nornicotine when compared to a leaf from TN90 LC.

5. The harvested leaf of claim **4**, wherein said reduced amount of nornicotine is reduced in a smoke stream produced from burning said leaf.

6. A tobacco product, comprising tobacco material prepared from the tobacco plant, or part thereof, of claim **2**.

7. The tobacco product of claim **6**, wherein said product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, leaf tobacco, shredded tobacco, cut tobacco, and chewing tobacco.

8. The tobacco product of claim **7**, wherein said product has a reduced amount of nornicotine when compared to a tobacco product made from TN90 LC.

9. A seed of tobacco cultivar NCBEX1MS, a tobacco cultivar essentially derived from said tobacco cultivar NCBEX1MS, or a tobacco hybrid produced from tobacco cultivar NCBEX1MS, wherein said seed comprises SEQ ID NO: 1 and SEQ ID NO: 2, a representative sample seed of said cultivar NCBEX1MS is deposited with the ATCC under ATCC Accession No. PTA-11719, wherein said tobacco cultivar NCBEX1MS, said tobacco cultivar essentially derived from said tobacco cultivar NCBEX1MS, and said tobacco hybrid produced from said tobacco cultivar NCBEX1MS comprises a percent nicotine conversion less than about 2.5%.

10. A tobacco plant, or a part thereof, produced by growing the seed of claim **9**, wherein said part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

11. A harvested leaf of the tobacco plant of claim **10**.

12. The harvested leaf of claim **11**, wherein said leaf has a reduced amount of nornicotine when compared to a leaf from TN90 LC.

13. The harvested leaf of claim **12**, wherein said reduced amount of nornicotine is reduced in a smoke stream produced from burning said leaf.

14. A tobacco product, comprising tobacco material prepared from the tobacco plant, or part thereof, of claim **10**.

15. The tobacco product of claim **14**, wherein said product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, leaf tobacco, shredded tobacco, cut tobacco, and chewing tobacco.

16. The tobacco product of claim **15**, wherein said product has a reduced amount of nornicotine when compared to a tobacco product made from TN90 LC.

17. An F_2 progeny plant of tobacco cultivar NCBEX1F, wherein said F_2 progeny plant comprises SEQ ID NO: 1 and

SEQ ID NO: 2, a representative sample seed of said cultivar is deposited with the ATCC under ATCC Accession No. PTA-11718 wherein said F_2 progeny plant comprises a percent nicotine conversion less than about 2.5%.

18. An F_2 progeny plant of tobacco cultivar NCBEX1MS, wherein said F_2 progeny plant comprises SEQ ID NO: 1 and SEQ ID NO: 2, a representative sample seed of said cultivar is deposited with the ATCC under ATCC Accession No. PTA-11719 wherein said F_2 progeny plant comprises a percent nicotine conversion less than about 2.5%.

19. The F_2 progeny plant of claim **17**, wherein said F_2 progeny plant is male sterile (MS).

20. The F_2 progeny plant of claim **18**, wherein said F_2 progeny plant is male sterile (MS).

21. The tobacco product of claim **6**, wherein said tobacco product is prepared from said tobacco cultivar NCBEX1F or a tobacco hybrid derived from said tobacco cultivar NCBEX1F.

22. The tobacco product of claim **14**, wherein said tobacco product is prepared from said tobacco cultivar NCBEX1MS or a tobacco hybrid derived from said tobacco cultivar NCBEX1MS.

23. The tobacco seed of claim **1**, wherein said seed is from tobacco cultivar NCBEX1F or a tobacco hybrid produced from tobacco cultivar NCBEX1F.

24. The tobacco product of claim **6**, wherein said tobacco product comprises tobacco material from said tobacco cultivar NCBEX1F or said tobacco hybrid produced from said tobacco cultivar NCBEX1F.

25. The tobacco product of claim **6**, wherein said tobacco cultivar NCBEX1F, said tobacco cultivar essentially derived from said tobacco cultivar NCBEX1F, and said tobacco hybrid produced from said tobacco cultivar NCBEX1F comprises a percent nicotine conversion less than about 2%.

26. The tobacco seed of claim **9**, wherein said seed is from tobacco cultivar NCBEX1MS or a tobacco hybrid produced from tobacco cultivar NCBEX1MS.

27. The tobacco product of claim **14**, wherein said tobacco product comprises tobacco material from said tobacco cultivar NCBEX1MS or said tobacco hybrid produced from said tobacco cultivar NCBEX1MS.

28. The tobacco product of claim **14**, wherein said tobacco cultivar NCBEX1MS, said tobacco cultivar essentially derived from said tobacco cultivar NCBEX1MS, and said tobacco hybrid produced from said tobacco cultivar NCBEX1MS comprises a percent nicotine conversion less than about 2%.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,096,864 B2
APPLICATION NO. : 13/407421
DATED : August 4, 2015
INVENTOR(S) : Lewis et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification:

Column 7, Line 13: Please correct "includes an F_t progeny"
to read -- includes an F_1 progeny --

Column 18, Line 27: Please correct "e4e5le4e5"
to read -- e4e5\e4e5 --

Signed and Sealed this
Fifteenth Day of March, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office